FOOD ACCESSORY FACTORS (VITAMINS) IN BACTERIAL CULTURE. II.

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In a previous paper[1] I called attention to the rôle that hemoglobin plays as a possible food accessory substance in the culture of hemophilic bacteria. It was shown that while these organisms in hemoglobin mediums attain a certain development, a much greater growth occurs when fresh animal or plant tissue is added to the medium or another bacterium is allowed to grow on the same plate. It would seem that there are at least these two food factors which tend to enhance the growth of the hemophil. These facts naturally suggest that the effect of the tissues on the plate cultures is dependent on substances comparable or analogous to the so-called vitamin substances that play an important rôle in animal metabolism.

The problem of finding substances which might enhance the growth of other bacteria has been undertaken. In the tests recorded in this article, the following bacteria were used: B. coli, B. typhosus, B. diphtheriae, Streptococcus hemolyticus, Staphylococcus aureus, Blastomycetes, Sporotrichum schenckii, Streptothrix, B. pyocyaneus, and B. prodigiosus.

In the first place, the effect of hemoglobin on these various organisms was tested. It is known that only a few organisms besides B. influenzae absolutely require hemoglobin. I had occasion some time ago[2] to review the facts. They are all small gram-negative bacilli. Other virulent bacteria like streptococci, pneumococci, meningococci, and gonococci grow well on rich blood medium to which other animal fluids like serum or ascites fluid is added. In my experience in growing the various bacteria of this type I have not noted that hemoglobin fluids are superior to other body fluids in this respect.

The ten organisms were inoculated on blood agar and serum agar 1 to 5 in each case and the series allowed to grow side by side, 8 succes-

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[1] Davis: Jour. Infect. Dis., 1917, 21, p. 392.
[2] Davis: Jour. Am. Med. Assn., 1915, 64, p. 1814.

sive transfers being made at intervals of 2 to 3 days. They all grew well and the two series yielded growths that to the eye were quite alike in amount. Unlike the true hemophils then these organisms are not apparently specifically stimulated to grow through the addition of hemoglobin to ordinary mediums.

Pure hemoglobin 1% was next added to tubes of distilled water which were inoculated with the various organisms. In such tubes the organisms vary in the amount of growth that develops. B. diphtheriae and streptococci did not grow well. It was doubtful whether any growth occurred in the tubes and continuous transfers with these bacteria was not possible. The other bacteria all evidently showed some growth but it was not abundant and did not compare with growth on ordinary mediums containing peptone and albumin.

Since the presence of typical vitamin substances causing definite effects in man and animals exist in the bran of such grains as rice and wheat, tests were now made to determine whether or not the presence of these bodies in any way influenced bacterial growth. The medium consisted of 0.5% NaCl and 1% agar to which was added 5% by weight of each of the following substances: Polished rice flour, unpolished rice flour, pure white wheat flour, and whole wheat flour. The medium was heated at 100 C. for from 1-2 hours and then without filtration tubed and slanted. The 10 organisms were inoculated into each of the mediums, placed in the incubator, and each day the growth was carefully observed as to quantity. Transfers were made at intervals of 3 or 4 days through 10 generations for the purpose of testing the viability of the organisms when thus transferred; such observations actually extending over a period of 38 days.

All of the organisms grew on the mediums and none died out during the period of observation. They grew equally well on all 4 varieties, no appreciable differences being noted. The growth was not profuse, on the whole being less than on ordinary plain meat extract agar which was used as a control.

In the second series, polished rice, unpolished rice, and wheat bran were each added to ordinary plain meat extract agar and the organisms tested as before, transplants being made every few days. Careful observation from day to day on these cultures did not reveal any appreciable increase in the profuseness of the growth as compared with the growth in tubes not containing the grain products. In this respect the 2 sets of experiments were quite in accord.

B. diphtheriae and S. hemolyticus grew poorly on all the mediums containing grain products. The fungi — Sporothrichum schenckii, blastomycetes, and a pathogenic streptothrix, on the other hand, grew about equally well on all the varieties of medium. The growth was quite rapid, profuse and especially was pigment formation abundant and intense. In this respect these mediums are far superior to ordinary agar and even, especially in intensity of pigment production, to Sabouraud's 4% maltose agar. No differences were noted, however, between the growth on the medium containing the bran or hulls and that not containing it. Therefore, it would seem that the vitamin substances of grains, important in the prevention of beriberi and related diseases, are not of significance in promoting or influencing the growth of the organisms studied.

Another series of experiments were made using mediums containing the sprouted grain as a constituent. Rice and wheat were moistened and allowed to germinate until sprouts were about 1 cm. or more long. They were now dried and ground into a fine flour and then used in 5% solution exactly as was the flour in the previous experiments. This sprouted grain medium was decidedly superior to the unsprouted. All bacteria grew more rapidly and more profusely, including the fungi, which also yielded pigment in abundance on this medium. Several factors may be responsible for this. The sprouting process is associated with enzyme activity resulting in the solution of starch and in its change to sugars; also a change in the proteins with the formation of more soluble nitrogenous substances including amino-acids and related compounds. The presence of such substances no doubt furnishes a most excellent medium for bacteria. It has been observed by Holst and Froelich, Funk, and others that sprouted grain will prevent certain deficiency diseases simulating scurvy in animals and which results from feeding dry grains like oats, wheat, etc.; it is suggested that in the germination of the seed, substances are formed which act as antiscorbutics. The question therefore arises whether such factors may not play a rôle in the results I obtained and, at least in part, possibly explain the increased development of the organisms on this medium.

Hopkins[3] has observed that vitamins are substances readily adsorbed, and more recently Dorothy Lloyd[4] has emphasized this in connection with observations on the growth of meningococci; filtration

[3] Jour. Physiol., 1912, 44, p. 425.
[4] Jour. Path. and Bacteriol., 1916, 21, p. 113.

through filter paper or cloth will largely remove these bodies from solutions, but glass wool does not appear to adsorb them.

In the experiments in which sprouted wheat grain was used, portions of the medium were filtered several times through layers of filter paper and other portions were not. It was noted that on the whole the unfiltered part yielded decidedly the more abundant growth. It was especially noticeable in the cultures of the more profusely growing organisms like B. coli and staphylococci, less noticeable though evident in the case of cultures of B. diphtheriae and streptococci. The filtered part from the sprouted grain yielded growths of bacteria which though less profuse than on the unfiltered medium was without question superior to that obtained on mediums made with the unsprouted grain. This was evident in all the tubes without exception and is no doubt due to the presence of constituents rendered soluble by the sprouting process. These facts are therefore quite in accord with the observations on the adsorbability of certain of the growth factors in the medium and would point to vitamin substances in the sprouted grain medium as playing a rôle in growth stimulation.